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EFFECT OF TWO BIOLOGICAL SUBSTRATES ON THE PRODUCTIVITY OF THREE GENERATIONS OF MYCELIUM (PLEUROTUS OSTRAETUS) IN DALOA (IVORY COAST)

Kouadio Serge N'GONIAN *, Affoué Sylvie Rachelle N'GUESSAN, Konan Elie YOBOUET and Mambé Auguste-Denise BOYE

Laboratory for Agricultural Production Improvement; Agroforestry Department, Jean Lorougnon GUEDE University, Daloa. P.O. Box 150, Daloa, Côte d'Ivoire.

ORCID Details

Kouadio Serge N'GONIAN: <https://orcid.org/0009-0001-6938-2209>

Affoué Sylvie Rachelle N'GUESSAN: <https://orcid.org/0009-0009-08542166>

Konan Elie YOBOUET: <https://orcid.org/0000-0000-0000-0000>

Mambé Auguste-Denise BOYE : <https://orcid.org/0009-0006-2963-3655>

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ABSTRACT

Mushroom cultivation offers a promising alternative for food security and the utilization of agricultural byproducts in Côte d'Ivoire. However, access to high-performance substrates limits productivity. This study aims to evaluate the effect of two local substrates on the growth and fruiting performance of *Pleurotus ostreatus* in Daloa (Côte d'Ivoire). An experimental design was established to test two substrates made from teak sawdust and various amendments (S1 composted for 45 days and S2 composted for 3 days) combined with three mycelial generations (F1, F3, F4). The parameters measured included colonization time, fruiting body yield, biological efficiency, and success rate. The rate of vegetative colonization was faster with generation F1 (32 to 34 days) compared to generations F3 and F4 (40 days), although the type of substrate did not show a significant effect on this parameter ($P > 0.05$). In contrast, the success rate increased with each subculture, reaching 100 % in F4 compared to 70 % in F1. Regarding fruiting, substrate 1 combined with the F3 generation (S1F3) performed best, with a maximum yield of 231.98 g/bag and a biological efficiency of 23.20 %. Although advanced subcultures (F4) ensure excellent mycelial growth, combining the S1 substrate with the F3 generation represents the optimal physiological compromise for maximizing the productivity of *Pleurotus ostreatus* in Daloa.

Keywords: Mycelium Generations, Colonization Time, Yield, Biological Efficiency

1 INTRODUCTION

African agriculture faces the dual challenge of ensuring nutritional security for its populations and reducing waste from agricultural production. In Côte d'Ivoire, food security and the diversification of income sources are major

socioeconomic issues, sparking growing interest in mushroom cultivation as an alternative source of high-biological-value protein (Oyetayo, 2011). Among cultivated species, the oyster mushroom (*Pleurotus ostreatus*) stands out for its ecophysiological plasticity, its ability to break down lignocellulosic material, its rapid growth, and the simplicity of its cultivation techniques (Chang & Miles, 2004 ; Sánchez, 2004).

In the Central-Western region of Côte d'Ivoire, the city of Daloa is a major agricultural hub that generates significant quantities of organic residues (coffee husks, cocoa shells, sawdust, and corn cobs), which are frequently burned or discarded without being fully utilized (Sánchez, 2004; Kouassi et al., 2018). Using these byproducts as a cultivation substrate presents an opportunity to reduce production costs and promote a local circular economy (N'Guettia et al., 2020).

However, local producers face two major constraints: access to high-performance growing media and dependence on frequent resupply of high-quality seeds (Philippoussis, 2009). To mitigate this dependence, the successive reuse of mycelium across multiple generations (F1, F2, F3) through local replanting is often practiced. Nevertheless, these successive replantings frequently lead to a decline in mycelial vigor or yield losses due to the degeneration of the biological material, a phenomenon that remains poorly documented under the region's microclimatic conditions (Mizuno, 1996 ; Royse et al., 2017, Tchougang et al., 2018).

Consequently, a central question arises: to what extent does the nature of the local substrates available in Daloa influence the productivity of *Pleurotus ostreatus*?

To address this issue, this study aims to contribute to improving the productivity of *Pleurotus ostreatus* over three successive generations on two local substrates in Daloa. More specifically, the study will determine the time required for mycelium generations to colonize the substrates, evaluate the yield and biological efficiency of the substrates, and determine the success rate of the mycelium generations.

2 MATERIALS

2.1 Study Site

This study was conducted at Jean Lorougnon GUEDE University (Daloa), located between 6.8944617 degrees north latitude and 6.4411233 degrees west longitude in Ivory Coast.

2.2 Biological Materials

The plant material studied consisted of seeds from three generations of mushrooms of the genus *Pleurotus ostreatus* that were 15 days old (F3 and F4) and more than 18 days old (F1), purchased from "Jardin Eden Park" (Figure 1).



Figure 1: Bottles containing *Pleurotus ostraetus* spores

3 METHODS

3.1 Preparation of Growing Media

Two types of growing media were prepared using various composted materials (Table 1).

Table 1: Composition of Growing Media

Components	Substrate 1		Substrate 2
Teak sawdust	70 kg	100 kg	100 kg
Rice bran	7 kg	5 kg	3 kg
Calcium carbonate	0.7 kg	40 kg	0.5 kg
Brown sugar	-	-	0,1 kg
Peelings (banana, cassava, etc.)	-	-	20 kg
Jeemp1 / water	-	-	1 /60 l
Herbal tea / water	-	-	1 /60 l
Jeemp 5 / water	-	-	1/2 l
Turning / days	4		1
Composting time	45 days		3 days

Jeemp 1 and herbal tea are bio-activators that accelerate the maturation and enrichment of compost. Jeemp 5 is an organic pest repellent that is diluted depending on the nature of the infestation.

3.2 Filling the Growing Bags

During this step, sixty double-lined, heat-resistant plastic bags (19 × 28 cm) were used, at a rate of thirty bags per substrate. They were then filled with the prepared substrates, at a rate of 1 kg of substrate per bag (Figure 2).

**Figure 2: Substrates sealed in heat-resistant plastic bags after filling**

3.3 Sterilization of Culture Substrates

Sterilization of the substrates began by placing 20 cm³ of water in a barrel containing a 25-cm-high stool (Figure 3A) on which the substrates were arranged. The barrel was then hermetically sealed and placed over a wood fire for three (03) hours at 100 °C (Figure 3B). After 3 hours of heating, the fire was removed. The substrates were then removed from the barrel and stored in a room that had been previously disinfected. To prevent any contamination, hand washing with alcohol or ether was required when handling the sterilized substrates.



Figure 3: Sterilization setup

A: Arrangement of substrates inside the barrel; B: Barrel containing the substrates placed over a wood fire

3.4 Inoculation of the substrates

Inoculation was performed 24 hours after sterilization of the substrates using three generations of *Pleurotus ostreatus* spawn (F1: Mycelium from the first generation; F3: Mycelium from the third generation; F4: mycelium from the fourth generation). The process involved seeding the substrates with the spawn using a plastic spoon, at an average of 17 g of spawn per substrate. The culture substrates were then sealed with PVC bands to prevent contamination (Figure 4).



Figure 4: Inoculation of the substrates

3.5 Incubation

The inoculated substrates were incubated for an average of 40 days at a temperature ranging from 27 to 34 °C to promote better mycelial growth (Figure 5A). After 40 days of incubation, the culture substrates were transferred to the mushroom house using wheelbarrows once the primordia had appeared. The mushroom house is a greenhouse-like structure characterized by diffused light, high humidity, and a moderate temperature (18–25 °C during the day) (Figure 5B).



Figure 5: A- Incubation room; B- Mushroom growing room

3.6 Unwrapping and Harvesting

The substrates were unwrapped after 3 days of watering with ice-cold water. This involved removing the PVC bands used to seal the substrates during inoculation (Figure 6A). Harvesting was performed every two (02) days after the first mushrooms appeared, for a period of five weeks. The substrates were then sprayed with running water using a sprayer to promote the growth of new mushrooms after each harvest (Figure 6B). In fact, the watering helped maintain the substrates' humidity between 60 and 70 °C, which is conducive to oyster mushroom development.



Figure 6: Cultivation Room

A: Unpacked substrates; B: Spraying the substrates after harvest

3.7 Data Collection

The parameters observed were the time required for substrate colonization, substrate yield, biological efficiency of the substrates, and the success rate of mycelium generations.

3.8 Substrate colonization time

Substrate colonization time is the time it takes for the mycelium to fully colonize the inoculated substrate (in number of days). It is calculated using the formula below:

$$Dc \text{ (jrs)} = (\sum \text{ Migration time}) / (\text{Number of bags per}$$

DC: Colonization duration; days: Days

3.8.1 Substrate yield

Yield is the mass, in grams, of mushrooms harvested per bag from each substrate, taking the treatment into account (Oei, 1993).

$$Y (g) = \sum \text{Yields per harvest}$$

3.8.2 Biological efficiency of substrates

Biological efficiency is the ratio of the mass of fresh mushrooms harvested to the mass of fresh substrate. To determine this, the quantity of mushrooms per bag on each substrate was measured at each harvest, taking into account the generations (Lalou, 2016). Consecutive harvests were therefore conducted for this purpose. Biological efficiency is a measurement tool for determining the growth potential of oyster mushrooms. It allows for the evaluation of how the cultivated species responds to the substrate.

$$BE (\%) = \frac{FW}{FWS} \times 100$$

BE: biological efficiency; FW: fresh weight of the mushroom; FWS: fresh weight of the substrate

3.8.3 Success rate

The success rate (SR) was determined by counting the number of uninfected bags, multiplying that number by 100, and dividing it by the total number of bags per treatment. This calculation was performed for each generation and for each substrate using the following formula.

$$SR (\%) = (\text{Number of bags that actually migrated}) / (\text{Total number of bags per treatment}) \times 100$$

3.9 Statistical Analyses

The data were entered using Microsoft Excel 2016, and analyses of variance (ANOVA) were performed using Statistica version 7.1 at a significance level of 5 % (0.05). In cases of significant differences, a Tukey HSD post-hoc test was conducted to compare the means.

4 RESULTS

4.1 Substrate Colonization Time by Mycelium Generations

Table 2 shows the time required for substrate colonization by mycelium generations. Statistical analysis revealed no significant difference between the two types of substrates ($P > 0.05$). However, the shortest colonization times were observed in the treatments, S1F1 (32 days), and S2F1 (34 days), compared to maximum times of 40 days for S1F4 and S2F3. The F1 generation therefore colonizes the culture substrates significantly faster than the F3 and F4 generations.

Table 2: Colonization time on the two types of substrate by generation

Treatments	Colonization time (days)
S1F1	32.000 ± 2.944 ^a
S1F3	39.500 ± 5.590 ^{ab}
S1F4	40.600 ± 6.406 ^b
S2F1	34.000 ± 2.160 ^a
S2F3	40.500 ± 4.787 ^b
S2F4	39.500 ± 6.717 ^{ab}
P-value	0.256

S1F1 : Substrate 1 inoculated with first-generation mycelia; S1F3: Substrate 1 inoculated with third-generation mycelia; S1F4: Substrate 1 inoculated with fourth-generation mycelia; S2F1 : Substrate 2 inoculated with first-generation mycelia; S2F3: Substrate 2 inoculated with third-generation mycelia; S2F4: Substrate 2 inoculated with fourth-generation mycelia. For each parameter in the same columns, values marked with the same letters are statistically equivalent.

4.1.1 Effect of substrates on the yield of cultured mycelium generations

Table 3 presents the yield by mycelium generation. Analysis of the data indicates that the highest yield was obtained with treatment S1F3 (231.98 g), while the lowest was observed for treatment S2F1 (167.4 g). Statistical analysis reveals that the means differ significantly between treatments ($P = 0.010$). These results indicate that the mycelium generations (F1, F3, and F4) yield higher yields on substrate 1 than on substrate 2, with treatment S1F3 providing the best overall performance.

Table 3: Yield over five weeks

Treatments	Yield (g)
S1F1	225.2 ± 25.2 ^a
S1F3	231.98 ± 19.94 ^a
S1F4	229.78 ± 20.5 ^a
S2F1	167.4 ± 17.4 ^b
S2F3	226.7 ± 13.37 ^a
S2F4	194.84 ± 21.63 ^{ab}
P-value	0.010

S1F1 : Substrate 1 inoculated with first-generation mycelia; S1F3: Substrate 1 inoculated with third-generation mycelia; S1F4: Substrate 1 inoculated with fourth-generation mycelia; S2F1 : Substrate 2 inoculated with first-generation mycelium; S2F3: Substrate 2 inoculated with third-generation mycelium; S2F4: Substrate 2 inoculated with fourth-generation mycelium; For each parameter in the same column, values marked with the same letters are statistically equivalent.

4.1.2 Biological efficacy of substrates as a function of the generations of mycelium Cultured

Figure 7 highlights the biological efficacy of the different treatments. Analysis of the data shows that treatments S1F1, S1F3, and S1F4 yield the highest biological efficacy on Substrate 1, with values of 22.52 %, 23.20 %, and 22.97 %, respectively. On substrate 2, treatment S2F3 yields the best performance with a biological efficiency of 22.67%, higher than that recorded for S2F4 (19.48 %). Statistical analysis reveals a significant difference among the treatments ($P = 0.01$). These observations indicate that the F3 generation exhibits greater biological conversion potential on both substrates compared to the other generations.

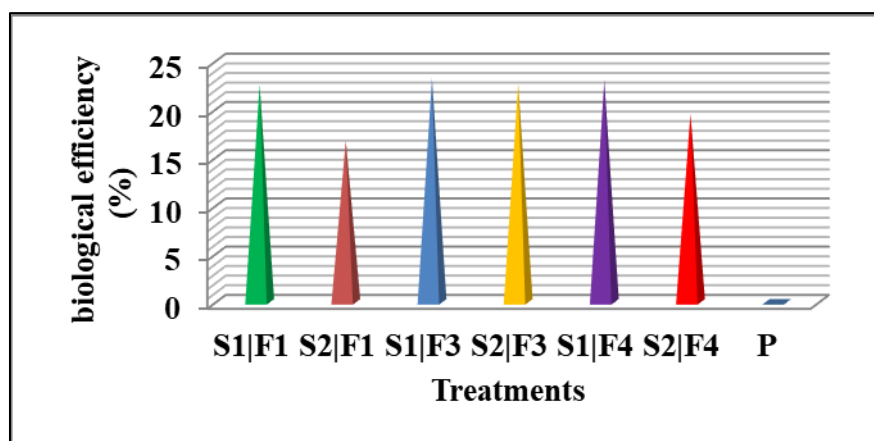


Figure 7: Biological efficiency of the substrates on the development of F1, F3, and F4 mycelia

F1: Mycelium from the first generation; F3: Mycelium from the third generation; F4: Mycelium from the fourth generation. S1 : Substrate 1 ; S2 : Substrate 2

4.1.3 Success Rate of Cultured Mycelium Generations

Figure 8 illustrates the success rate of the different generations of cultured mycelium. Data analysis indicates that the highest success rate was obtained with the F4 generation (100 %), while the lowest was recorded for the F1 generation

(70 %). Generation F3 shows an intermediate success rate of 9%. Statistical analysis confirms a significant difference among the mycelium generations ($P = 0.041$). These results indicate that the success rate varies significantly from one generation to the next, increasing gradually with each subculture.

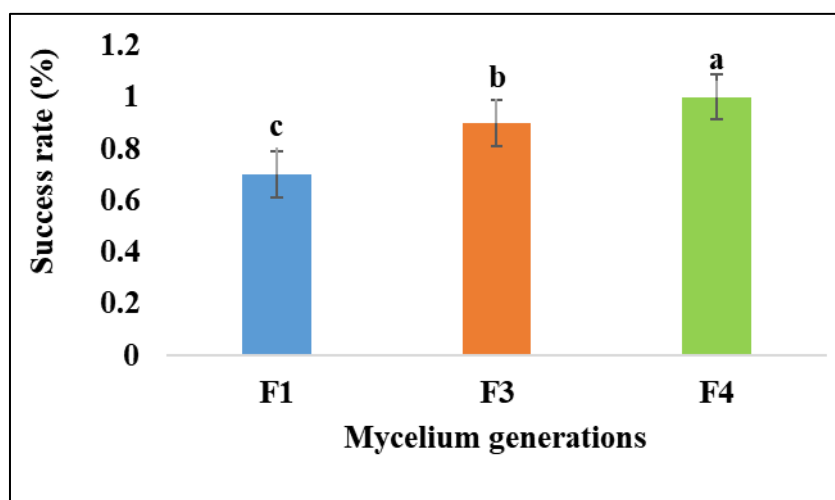


Figure 8: Success rates of the different mycelium generations

F1: Mycelium from the first generation; F3: Mycelium from the third generation; F4: Mycelium from the fourth generation.

5 DISCUSSION

Analysis of the colonization time showed that the type of substrate does not have a significant effect on the rate of mycelial invasion ($P > 0.05$). This result indicates that the two substrates tested have equivalent physicochemical properties (porosity, water retention, nutrient availability) capable of supporting optimal hyphal growth, consistent with the observations in (Grimm et al., 2021) regarding the adaptive plasticity of the mycelial network in response to various lignocellulosic materials. However, the colonization time varies significantly depending on the mycelium generations used. First-generation (F1) mycelia colonize the medium significantly faster (32 to 34 days) than the subsequent F3 and F4 generations, which take up to 40 days. Our results are consistent with those of Silliker et al. (2021) and Sun et al. (2022). The latter authors demonstrated that the decline in growth rate during successive subcultures reflects the phenomenon of mycelial senescence or degeneration. According to Navarro-Mendoza et. (2019) prolonged vegetative propagation, coupled with the accumulation of mitochondrial dysfunction and oxidative stress, leads to a loss of vigor and a reduction in the rate of hyphal extension. Furthermore, the gradual decline in the secretion potential of key extracellular enzymes (particularly laccases and cellulases) in older generations explains their delayed ability to effectively degrade and invade the substrate matrix (Sahu et al., 2020). F1 strains thus retain greater metabolic vigor and assimilation capacity than those of advanced-generation subcultures.

Furthermore, analysis of the yield results revealed significant variations depending on the substrate and the mycelium generation used ($P = 0.010$). Overall, Substrate 1 yields more abundant fruiting than Substrate 2 for all tested generations. Indeed, this superiority of Substrate 1 could be explained by its more balanced nutritional composition, particularly in terms of the digestibility of structural polysaccharides (cellulose, hemicellulose) and the availability of assimilable nitrogen factors that are critical for primordia formation and carpophore development ([Liu et al., 2023 ; Hoa, 2020). The highest yield was obtained with treatment S1F3 (23 1.98 g), while the lowest yield was observed in S2F1 (167.4 g). Interestingly, although F1-generation mycelia exhibited faster vegetative colonization, it was the F3 generation on substrate 1 that recorded the highest overall productivity. This discrepancy between the rate of substrate invasion and the harvested biomass suggests that the hyphal growth rate is not the sole predictor of fruiting yield (Bellettini et al., 2019). Furthermore, a slightly longer vegetative growth phase (as observed in F3) may allow the mycelial network to build up larger metabolic energy reserves and secrete a more diverse array of enzymes before primordiation begins (Owaid et al., 2017).

However, the decline in performance observed on substrate 2 for more advanced generations serves as a reminder that the accumulation of subcultures ultimately impairs the ability to assimilate nutrients from the medium (Sun et al., 2022). The S1F3 combination thus represents the optimal compromise between nutritional adaptation to the substrate and the strain's genetic potential for fruiting.

Furthermore, biological efficiency which reflects the fungus's ability to convert the substrate's dry matter into harvestable fungal biomass varies significantly depending on the substrate and the mycelium generation ($P = 0.01$). The

maximum values recorded on substrate 1 (notably with S1F3 at 23.20 %) confirm that this medium offers an ideal biochemical composition (carbon-to-nitrogen ratio, availability of soluble biopolymers) to support fruiting, consistent with the findings of Bellettini et al. (2019) and Atila, 2019).

A comparative analysis of the generations shows that the F3 generation consistently outperforms the others on both tested substrates (23.20 % on S1 and 22.67 % on S2). This result may be explained by the attainment of an optimal metabolic equilibrium at this stage of subculture. As noted by Owaid et al. (2017) and Liu (2023) after a few subcultures, the mycelium adjusts and reorganizes its lytic enzyme system (laccases, cellulases, and xylanases), thereby maximizing the degradation of the lignocellulosic matrix. However, the decline in biological efficiency observed beyond F3 (particularly in S2F4 at 19.48 %) highlights the limitations of continuous vegetative propagation. Furthermore, Navarro-Mendoza et al. (2019) and Sun et al. (2022) have shown that the decline in productivity in advanced generations results from the accumulation of oxidative stress and mitochondrial alterations linked to mycelial senescence. Thus, the F3 generation represents the optimal stage of physiological maturity for maximizing yield and biological efficiency before the onset of the deleterious effects of degeneration.

Analysis of the culture success rate shows a significant variation across mycelial generations ($P = 0.041$), with a steady increase from the F1 generation (70 %) to the F4 generation (100 %). In fact, the increase in the success rate over successive subcultures can be explained by the phenomenon of physiological adaptation or acclimatization of the mycelium to *in vitro* conditions (Grimm et al., 2021). Our results contradict those of Navarro-Mendoza et al. (2019) who demonstrated in their work that during the early stages of isolation (F1), the hyphal network undergoes a transfer shock due to the change in medium and exhibits greater sensitivity to environmental stressors, which reduces its establishment rate. However, our results are consistent with those of (Sahu et al., 2020). According to these authors, as subcultures progress (F3 and F4), the strain selects hyphal lines adapted to the synthetic or semi-synthetic substrate, optimizing its basic growth metabolism and strengthening its ability to overcome the initial inhibition phases. It is, however, essential to emphasize the difference between the success of vegetative establishment and final productivity. While the F4 generation exhibits the most reliable establishment rate (100 %), the literature shows that excessive replanting ultimately leads to genetic degeneration and a decline in fruiting capacity (Sun et al., 2022 ; Navarro-Mendoza et al., 2019). Thus, an advanced generation such as F4 guarantees excellent mycelial regrowth, although its potential for fruiting body yield must be evaluated in conjunction with the strain's overall metabolic vigor.

6 CONCLUSION

This study highlighted the critical interaction between substrate type and the number of mycelial subcultures on overall cultivation efficiency. Furthermore, the results showed that the colonization rate of *Pleurotus ostraetus* mycelium generations decreases over time. The results also revealed that the S1F3 treatment appears to be optimal for the healthy growth and development of the mushrooms (*Pleurotus ostraetus*). Furthermore, the success rate is higher with generation F4. Substrate 1 promotes better multiplication of mycelium generations compared to substrate 2. However, substrate 1 and the F3 mycelium generation can be recommended to mushroom growers to increase mushroom productivity and, consequently, their income. Thus, implementing the findings of this study will help promote mushroom cultivation in Africa (Ivory Coast) and achieve self-sufficiency in edible mushrooms in Ivory Coast.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

No competing interests exist.

Author Contributions

Kouadio Serge N'GONIAN: Study design, field data collection, interpretation of results, and manuscript drafting. Affoué Sylvie Rachelle N'GUESSAN and Konan Elie YOBOUET: Participation in data collection and manuscript review. Mambé Auguste-Denise BOYE: Scientific supervision and critical review of the manuscript.

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